International Journal of Environmental Bioremediation & Biodegradation, 2013, Vol. 1, No. 1, 1-5
Available online at http://pubs.sciepub.com/ijebb/1/1/1

DOI:10.12691/ijebb-1-1-1

Microbial Degradation and Decolorization of Reactive Orange Dye by Strain of *Pseudomonas Spp*

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Received August 23, 2013; Revised August 31, 2013; Accepted September 02, 2013

Abstract Azo dye Reactive orange was selected for decolorization and degradation studies by *Pseudomonas spp.* Optimization of parameters for dye decolorization were studied under static anoxic condition. Under optimized condition decolorization of Reactive orange by *Pseudomonas spp* was found to be 98% at 50mg/L within five hours in static anoxic condition. The optimum pH and temperature for the decolorization was 8.0 & 37°C respectively. The biodegradation was monitored by FTIR analysis. The results suggest that the isolated organism *Pseudomonas spp* as a useful tool to treat wastewater containing reactive dyes.

Keywords: *reactive orange, Pseudomonas, pH, temperature, static condition*

Cite This Article: Maulin P Shah, Kavita A Patel, Sunu S Nair, and A M Darji, "Microbial Degradation and Decolorization of Reactive Orange Dye by Strain of Pseudomonas Spp." *International Journal of Environmental Bioremediation & Biodegradation* 1, no. 1 (2013): 1-5. doi: 10.12691/ijebb-1-1-1.

1. Introduction

Rapid industrialization has necessitated the manufacture and use of different chemicals in day to day life [5,20]. The textile industry is one of them which extensively use synthetic chemicals as dyes. Wastewaters from textile industries pose a threat to the environment, as large amount of chemically different dyes are used. A significant proportion of these dyes enter the environment via wastewater [20]. Approximately 10,000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced annually, worldwide [26]. Pollution due to textile industry effluent has increased during recent years. Moreover, it is very difficult to treat textile industry effluents because of their high BOD, COD, heat, color, pH and the presence of metal ions [4]. The textile finishing generates a large amount of waste water containing dyes and represents one of the largest causes of water pollution [6], as 10- 15% of dyes are lost in the effluent during the dyeing process [36]. The traditional textile finishing industry consumes about 100 liters of water to process about 1 Kg of textile material. The new closed-loop technologies such as the reuse of microbial or enzymatic treatment of dyeing effluents could help reducing this enormous water pollution [1]. Azo dyes have been used increasingly in industries because of their ease and cost effectiveness in synthesis compared to natural dyes. However, most azo dyes are toxic, carcinogenic and mutagenic [24]. Azo bonds present in these compounds are resistant to breakdown, with the potential for the persistence and accumulation in the environment [31]. Several physico-chemical techniques have been proposed for treatment of colored textile effluents. These include adsorption on different materials, oxidation and precipitation by Fenton's reagent, bleaching with chloride or ozone photo degradation or membrane filtration [27]. All these physical or chemical methods are very expensive and result in the production of large amounts of sludge, which creates the secondary level of land pollution. Therefore, economic and safe removal of the polluting dyes is still an important issue. Bioremediation through microorganisms has been identified as a cost effective and environment friendly alternative for disposal of textile effluent [10,25]. In recent years a number of studies have focused on some microorganisms capable of degrading and absorbing dyes from wastewater. A wide variety of microorganisms are reported to be capable of decolonization of dyes [2,3,8,9,12,14,15,17,18,19,28,32,35]. The present study deals with the isolation of textile dyes degrading bacterium from a dyes contaminated environment, its ability to degrade reactive dyes.

2. Materials & Methods

2.1. Media and Chemicals

All media components and chemicals used in the present study were of analytical grade and purchased from Hi-Media Laboratories (Mumbai, India). The textile dyes, Reactive orange used for decolorization study was a gift from local textile industry, Ankleshwar, Gujarat, India.

2.2. Microorganism and Culture Conditions

Dye industry effluent contaminated soil, sewage, and dye waste were subjected for acclimatization to reactive orange, in the basal nutrient medium, nutrient broth. The

most promising bacterial isolate was used for further dye degradation studies. The culture was identified as *Pseudomonas spp*. Pure culture was maintained on the nutrient agar slants. Composition (g/L) of nutrient agar and broth used for decolorization was peptic digest of animal tissue 5, NaCl 5, Beef extract 1.5, Yeast extract 1.5 and pH 7.4 +/- 0.2.

2.3. Identification of the Culture

Identification of the isolate was done by Bergey's Manual of Determinative Bacteriology (2000) [11,30]. *Pseudomonas spp*. was grown for 24hr. at 37°C on nutrient agar. Inoculum 10% ($O.D_{600}$ 1.0) was used throughout the study. The isolate was inoculated in nutrient broth to study the decolorization performance of the culture. The dye was filter sterilized by using 0.2µM cellulose acetate paper filter and added after sterilization of medium throughout the study. The dye (50mg/L) was added immediately and incubated at static condition at 37°C. The aliquot (3mL) of culture media was withdrawn at different time intervals and centrifuged at 6,200g for 10 min. Decolorization with respect to time was monitored by measuring the absorbance of the culture at λmax of the dye at 542nm. Weight was recorded in mg/mL and change in pH was also recorded.

2.3.1. Decolorization at Different Dye Concentration

In order to examine the effect of initial dye concentration on the decolorization in static condition 50-250 mg/L of reactive orange was added to the nutrient broth inoculated with 10% of *Pseudomonas spp.* O.D 600 and incubated at 37°C under static condition. The percentage of decolorization was measured after 13h. All decolorization experiments were performed in three sets. Abiotic controls (without microorganisms) were always included. The percentage of decolorization was calculated.

$$Decolorization(\%) = \frac{Inital\ absorbance - observed\ observance}{Initial\ observance} \times 100$$

$$Average\ decolorization\ rate = \frac{C \times \%D \times 1000}{100 \times t}$$

Where, C = initial concentration of dye (mg/L),
% D=Dye decolorization (%) after time t [22].

2.3.2. Effect of pH on Dye Decolorization

Nutrient broth of different pH [3-9] was inoculated with 10% inoculums and incubated at 37°C under static condition. The dye concentration was 50mg/L. All decolorization experiments were performed in three sets. An abiotic control (without microorganisms) was always included. The percentage decolorization was measured. pH was adjusted by using 1N HCl & 1N NaOH.

2.3.3. Effect of Temperature on Dye Decolorization

Nutrient broth of pH 7.0 was inoculated with 10% of inoculums and filter sterilized dye at 50 mg/L was added after sterilization. The broth was incubated at 22, 25, 27, 30, 37, 40, 42 and 45°C. The experiment was carried out in triplicate. An abiotic control (without microorganisms) was always included. The percentage decolorization was measured.

2.3.4. Effect of Carbon and Nitrogen Sources

To study the effect of carbon and nitrogen sources on decolorization of reactive orange, semi synthetic medium having following composition was used: (g/L) $(NH_4)_2SO_4$ 0.28, NH_4Cl 0.23, KH_2PO_4 0.07, $MgSO_4$. $7H_2O$ 0.04, $CaCl_2$. $2H_2O$ 0.022, $FeCl_3$. $6H_2O$ 0.005, Yeast extract 0.2, NaCl 0.15, $NaHCO_3$ 1.0 & 1ml/L of trace elements solution containing (g/L) $ZnSO_4.7H_2O$ 0.01, $MnCl_2$ $4H_2O$ 0.1, $CuSO_4$ $5H_2O$ 0.392, $CuCl_2$ $6H_2O$ 0.248, NaB_4O_7 $7H_2O$ 0.177 & $NiCl_2$ $6H_2O$ 0.02 with different carbon and nitrogen sources. (1% each) such as glucose, sucrose, lactose and starch, yeast extract, peptone, malt extract, meat extract & urea respectively. 50 mg/L of the dye concentration used. Filter sterilized dye was added after sterilization of the medium and after inoculation of the isolate.

3. Results & Discussion

3.1. Isolation & Identification of Dye Decolorizing Bacteria

Dye industry effluent contaminated soil, sewage, and dye waste were subjected for acclimatization to Reactive orange in the basal nutrient medium, nutrient broth. Isolation of bacteria was carried out by the enrichment technique using nutrient broth and Reactive orange as source of carbon and nitrogen that has rapid decolorization capacity. Decolorization occurred only when a carbon and nitrogen sources were available for growth. Microorganism showing maximum decolorization in less time was selected & using Bergy`s Manual of Determinative Bacteriology (2000) was identified as *Pseudomonas spp*. The isolate showed ability to decolorize Reactive orange within 5 h at a dye concentration 50 mg/L. The absorbance peaks in the visible region disappeared indicating complete decolorization [13]. In the UV spectra the peak at 542 nm was replaced by new peak (Figure 1).

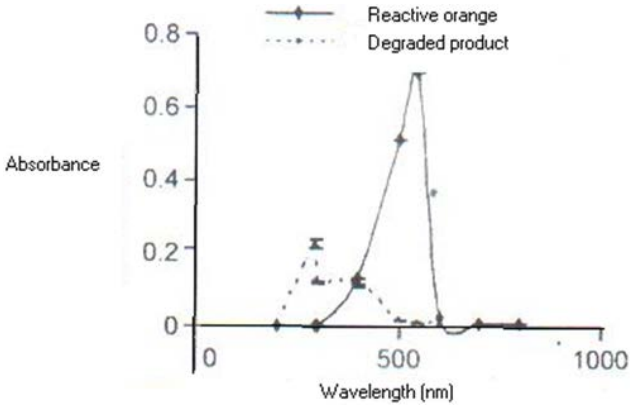

Figure 1. UV spectrophotometric analysis of the dye reactive orange and its degradation product

3.2. Effect of Physicochemical Conditions on the Decolorization Performance

The effect of various physicochemical conditions such as pH, temperature, dye concentration, effect of carbon and nitrogen sources on decolorization of Reactive orange by the isolate was studied in detail. All parameters were studied at 37°C under static condition. 10% inoculums with $O.D_{600}$ 1.0 were used at 50 mg/L dye concentration.

3.2.1. Effect of pH

Bacterial cultures generally exhibit maximum decolorization at pH values near 7-8. In the present study the culture exhibited decolorization activity in the range of pH 5-8. At pH 3 and 4 decolorization observed was 15 and 38% respectively. The isolate showed more or less constant decolorization from pH 5 to 8. Maximum (98%) being at pH 8 (Figure 2). The isolate exhibited decolorization ability in the range of pH 5-8. This is in accordance with previous reports; *Pseudomons aeruginosa* BCH exhibited best decolorization at pH 8 [29].

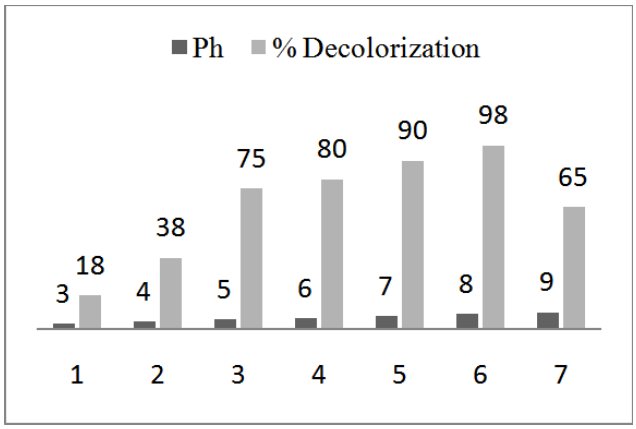

Figure 2. Effect of pH on decolorization

3.2.2. Effect of Temperature

Temperature is another very important parameter for treatment of wastewater. Selected isolate is mesophilic bacteria because it showed better decolorization in the temperature range of 25 to 37 °C. Similar results were also reported by Guo *et al*. (2008). The mesophilic range is traditionally used [33] since it is generally thought that maintaining high temperature would be uneconomical, while degradation within the psychrophilic range is too slow. *Pseudomonas spp.* showed strong decolorizing activity from 27°C to 37°C (Figure 3). Although a lag phase was observed and the decolorization rate was comparatively low at 22°C, the decolorization extent increased to a similar level from 27°C to 37°C. Decolorizing activity was significantly suppressed at 42°C, which might be due to the loss of cell viability or deactivation of the enzymes responsible for decolorization at 42°C [7,21]. The isolate showed complete decolorization at 30, & 37°C but rapid decolorization was observed at 37°C (Figure 3). At 42 & 45°C there was no decolorization. Hence temperature optimum for Reactive orange was found to be 37°C. This may be owing to a greater production of enzymes and optimal growth conditions of the isolate for its dye decolorizing ability. The decolorization at this optimum temperature may be owing to higher respiration and substrate metabolism. This also demonstrate that decolorization of the dye was through microbial reaction which relies on optimal temperature and not by adsorption. At 42 & 45°C there was no decolorization.

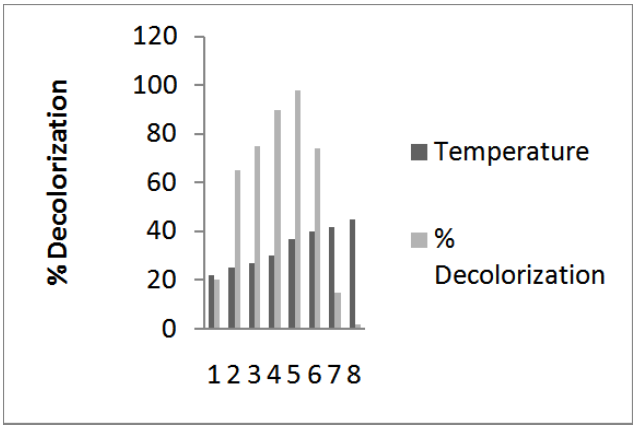

Figure 3. Effect of temperature on decolorization

3.2.3. Effect of Initial Dye Concentration

Decolorizing ability of the culture in the present study increased with increase in dye concentration from 50-250 mg/L (Figure 4). The isolate could decolorize 95% of 50 mg/L of the dye in 5 h whereas it took 48 h to decolorize 80% of 250 mg /L. the culture shows decolorizing ability up to 200mg/L at a faster rate after which the rate began decreasing. It has been proposed that efficiency of microbial decolorization through a combination of factors including the toxicity imposed by dye at higher concentration [23]. Thus, the isolate which could decolorize dye up to the reported dye concentration in wastewater, can be successfully employed for treatment of dye bearing industrial wastewater.

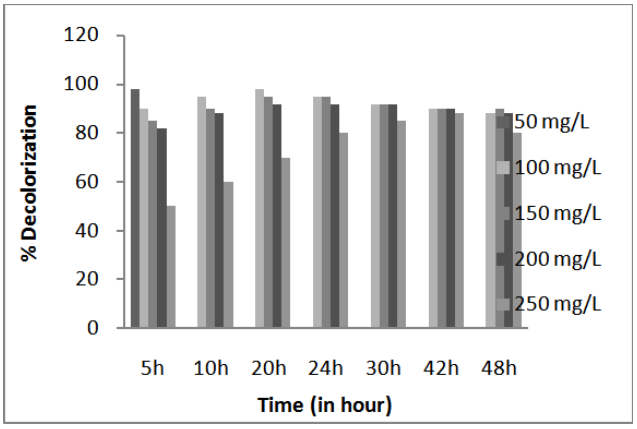

Figure 4. Effect of initial dye concentration

3.2.4. Effect of carbon and Nitrogen Sources

While trying to enhance decolorization performance of reactive orange, extra carbon and nitrogen sources was supplied in semi synthetic medium. There was no decolorization in the presence in the synthetic media. Percentage decolorization was maximum with purified substrate yeast 95% while 92 and 78% decolorization was showed when supplied with malt and meat extract respectively while less decolorization with other supplements of carbon and nitrogen source within 24h (Figure 5). Similar result was also reported for bacterium consortium RVM for decolorization of reactive violet 5 [22]. The culture showed moderate decolorization in the

presence of sucrose (50%) lactose (45%) and starch (55%) and decolorization in the presence of glucose was reported (62%). In addition supplying urea as a nitrogen source exhibited less decolorizing ability (38%). In contrast, addition of carbon sources seem to less effective to promote the decolorization probably due to the preference of the cells in assimilating the added carbon source seemed to be less effective to promote the decolorization probably due to the preference of the cells in assimilating the added carbon sources over using the dye compound as the carbon source.

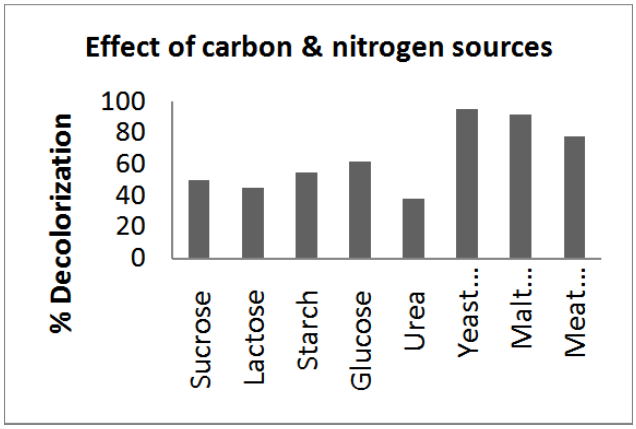

Figure 5. Effect of carbon & Nitrogen sources

3.3. FTIR Analyses

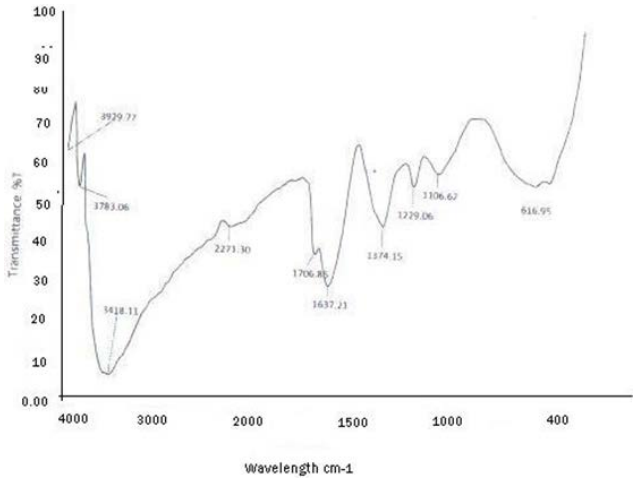

Figure 6. FTIR Spectrum of control dye

The FTIR spectrum of a control dye and extracted metabolites (24 hours) by *Pseudomonas spp.* was compared. The spectrum of the control dye (Figure 6) displayed a peak 3418.11 cm^{-1} for –NH stretching. The stretching between C N was reported at 2271.30 cm^{-1} and amide, 5-membered ring peak at 1706.86 cm^{-1}. The peak at 1637.21 cm^{-1} showed carbonyl stretching vibration. Peak at 1374.15 cm^{-1} showed unsaturated nitrogen compounds. Peak at 1229.06 cm^{-1} showed S=O stretching vibrations. The peak at 1106.62 cm^{-1} indicates the aromatic nature. The peak at 616.95 cm^{-1} showed hydrocarbon chromophore-C-H bending. The FTIR spectrum of 24 hours extracted metabolites of *Pseudomonas spp.* (Figure 7) showed a significant change in positions of peak, when compared to the control dye spectrum. A new peak at 1636.59 cm^{-1} represented –N=N-stretching vibration. The C-H deformation showed at 1398.13 cm^{-1}. The peak at 3408.37 cm^{-1} showed N-H stretching vibration.

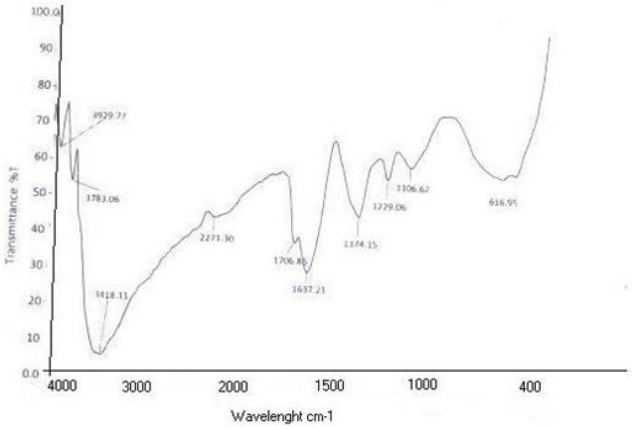

Figure 7. FTIR Spectrum of degraded dye

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